Pheromone-Based Attractant for Males of *Cactoblastis cactorum* (Lepidoptera: Pyralidae)

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ABSTRACT The cactus moth, *Cactoblastis cactorum* (Berg), is an invasive pest of *Opuntia* spp. Since its arrival in the Florida Keys in 1989, it has moved rapidly up the east and west coasts of Florida, threatening to invade the southwestern United States and Mexico. Female moths produce a sex pheromone that attracts male moths. In this study, we report on mating behavior observed in the laboratory and the identification of putative pheromonal chemical components based on mass spectral analysis of volatiles collected from virgin female moths and from solvent extraction of excised glands. Three candidate components, formulated on rubber septa in different release rates and ratios, were tested in laboratory olfactometer and flight tunnel experiments, and in field tests in areas with known feral populations of cactus moths. Lures formulated with the three-component blend of 54% (*Z*,*E*)-9,12 tetradecadien-1-ol acetate, 42% (*Z*,*E*)-9,12 tetradecadien-1-ol, and 4% (*Z*)-9- tetradecen-1-ol acetate were the most effective, although changes in the ratio of these components had little effect on lure efficacy. For field deployment, traps baited with synthetic lures with a 1 mg load of the three component blend captured equal or higher numbers of males than traps baited with two virgin females. Trapping systems using this pheromone-based attractant will be useful for population delineation in areas currently infested.

KEY WORDS sex pheromone, field trapping, lure, gland extracts, olfactometer

The cactus moth, *Cactoblastis cactorum* (Berg), is native to South America and was introduced into Australia in the 1920s for biological control of prickly pear cactus, *Opuntia* spp. This moth successfully controlled invasive *Opuntia* spp. and was subsequently introduced into Africa and numerous other areas, including the Caribbean Islands (Zimmermann et al. 2001). *C. cactorum* was first found in Florida in 1989 (Habeck and Bennett 1990), where it has become a pest of native *Opuntia* spp. (Bennett and Habeck 1992, Pemberton 1995). Cactus moths have moved rapidly up the east and west coasts of Florida, reaching the Florida panhandle and coasts of Georgia and South Carolina by 2002 (Hight et al. 2002). The moth will likely spread to other parts of North America and Central America either unaided or through the assistance of human activity. Recent hurricane activity in the Gulf of Mexico may further accelerate the spread of this pest. If the cactus moth continues to invade the southwestern United States and Mexico, the effects will be severe. In Mexico, cacti have been of special importance since ancient times and are featured in the history, economy, food, and cultural life of the country. To develop management strategies against this pest, such as the sterile insect technique and biological control, the development of a lure is critical. A trapping system based on a synthetic pheromone lure is required for population detection and delineation.

Typical of pyralid moths, female cactus moths produce a sex pheromone that is attractive to males, and traps baited with virgin females can be used to capture males (Hight et al. 2002). However, maintaining a trapping network with female-baited traps is less than ideal because of the short life span of the female moths, the cost of rearing the moths and placing them in traps, and the deleterious effect of inclement weather on female moth behavior. Also, to reduce the chance of an accidental introduction, female-baited traps should not be used in areas not yet infested with moths unless the females have been sterilized (Bloem et al. 2003). Therefore, research was conducted to

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identify the chemical components of the female-produced pheromone and to formulate a synthetic lure that can be used to detect invasions as well as to monitor population levels in areas with established populations.

Identification of the pheromonal components was based on mass spectral analysis of volatiles collected from virgin female moths and on analysis of chemicals obtained from solvent extraction of excised glands. Based on this identification, the pheromonal components were formulated on rubber septa and tested in areas with known populations of cactus moths. Additionally, laboratory flight tunnel experiments were conducted to determine the efficacy of the pheromone blend compared with volatiles from virgin female moths. We report here the identification of a multicomponent blend that is attractive to conspecific males.

Materials and Methods

Insects. Cactus moth adults were obtained as sexed pupae from the colony maintained at USDA–ARS in Tifton, GA; from larvae that were field-collected in Tallahassee, FL; and from a laboratory colony maintained at USDA–ARS in Miami, FL, using procedures reported previously (Carpenter et al. 2001). The laboratory colony maintained in Miami was reared on *O. ficus-indica* L. Miller variety Lynnwood and variety Santa Ynez (Rivenrock Gardens, Nipomo, CA). Newly emerged adults were collected each day and maintained in cages containing single sexes in separate rearing rooms at room temperature and ambient humidity until time of testing. The rearing rooms had windows to provide natural lighting and were supplemented with room lights set to a photoperiod of 12:12 (L:D) h. All females used for chemical analysis were 1–2 d old at time of testing.

Laboratory Mating Behavior. Tests were conducted to document mating behavior under laboratory conditions. All tests were conducted in bioassay rooms with windows so that natural light was available, and room lights were set to the same 12:12 (L:D)-h photoperiod as the rearing rooms. Observations on adult mating behavior (e.g., female "calling" or pheromone release posture, periodicity of calling, and time of mating) were made by videotaping individually held virgin males and females, as well as paired adults under an infrared light with a low light CCTV camera (BP330; Panasonic, Secaucus, NJ). For these observations, moths were placed in clear plastic 140-ml vials (4.8 cm ID by 8.6 cm long) with removable snap-top lids (Thornton Plastics, Salt Lake City, UT). A piece of aluminum window screen (4 cm diameter) was attached to the bottom of the clear vial with hot glue, a piece (7.62 by 7.62 cm) of filter paper (Whatman no. 1) was placed along the back wall of the vial to provide foot-holds for the moths, and the vial was inverted with the snap-lid becoming the floor. Moths were placed in the vials before the start of scotophase, and video output was recorded on a VCR throughout scotophase. Time of sunrise was obtained from the internet (http://aa.usno.navy.mil/data/docs/RS_OneDay.html).

Laboratory Bioassays. Y-tube olfactometers (35.5 cm total length by 2.54 cm ID; Analytical Research Systems, Gainesville, FL) were used to evaluate male response to calling females and to synthetic lures. Test substrates were placed in a small glass chamber (≈12.7 cm by 2.2 cm ID) with a downwind screen that was attached to one arm of the chamber. The second arm was attached to a clean air blank. Males were released individually at the far end of the tube before initiation of the calling posture of the female, and the relationship between onset of female calling and subsequent male behavior was recorded. Based on results reported in field trials (Hight et al. 2003), males were placed in the olfactometer at 15-min intervals starting 1.25 h and ending 0.5 h before sunrise. Up to six males were tested per time period in separate Y-tubes, with five males tested at each dosage/lure combination.

Flight tunnel bioassays were conducted using a Plexiglas wind tunnel (61 cm by 1.8 m) similar to that previously described (Heath et al. 1983), with modifications to allow use of an external odor source for the test substrate (Heath et al. 1993). Air was purified using charcoal-infused cloth and an air velocity of 0.22 m/s was maintained with a vacuum pump. Test substrates were placed in the upwind portion of the tunnel. Females were placed individually in cages (stainless steel mesh tea strainers, 5.08 cm width by 5.08 cm ID), and three cages containing females were placed in the upwind portion of the tunnel. To confirm that males were responding to volatile chemicals and not visual or auditory cues from live females, some tests used an external odor source (Heath et al. 1993). For this, three to five females were placed in a glass chamber (6.35 cm ID by 20.32 cm length) constructed of Pyrex glass with a glass frit inlet and a ground-glass joint outlet, a smaller version of the volatile collection system used for collection of chemicals from calling females (see below). In tests with synthetic blends (see below), a single lure was placed in the screen cage in the wind tunnel and one treatment was tested per day. Bioassays were conducted during the last hour of scotophase, and observations were aided by overhead red lights (<1 foot candles). Males were released individually at the downwind end of the tunnel and each male was transferred to the wind tunnel using a cylindrical plastic vial. The behavior of each individual male was observed for 3–5 min, and the response to the odor source was determined by two behavioral events: oriented flight (OF) and landing (LA). Flight tunnel bioassays of male response to live females or synthetic lures were replicated three times.

Pheromone Collection. Glands excised from calling females late in the scotophase were placed in a small amount of methylene chloride and chemicals extracted for 10 s. Initial chemical analyses yielded no candidate compounds. Therefore, 5 pmol of synthetic pheromone biosynthesis-activating neuropeptide (PBAN) in 2 μl of Ringers saline were injected to stimulate pheromone production and accumulation in abdominal glands as described by Teal et al. (1995).

Controls for these treatments were females injected with saline only. After an incubation period of 2 h, glands were excised from PBAN-treated females, placed in methylene chloride (5 μl/gland), and chemicals were extracted for 10 s. Gland extracts were stored at −20°C.

Volatile chemicals from calling females were collected using Super Q-packed adsorbent traps (Heath et al. 1983, Heath and Manukian 1992). Twenty to 50 virgin female cactus moths were placed in modified glass chambers with a water wick and a screen foothold for calling females and with or without a cactus pad. Purified air was humidified by passing the airstream through a bubbler and introduced into the chamber (1 liter/min). Volatile chemicals were collected for three consecutive 1-h time periods that included the end of scotophase. The volatile collection system consisted of a glass chamber (38.1 cm long by 11.4 cm ID) constructed of Pyrex glass with a glass frit inlet, a ground-glass joint outlet, and a multiport collector base to which the collector traps were connected. Air was purified using two in-line charcoal filters to remove organics from the air supply. The filters were made of 1.6-cm-OD (0.05-mm wall thickness) by 12.7-cm-long stainless steel tubing and contained 28-mesh activated charcoal (Alfa Products, Danvers, MA) packed between two 325-mesh stainless steel screens held in place by 1.6- to 0.64-cm Swagelok reducing unions. Connected in line with these filters was an adjustable single-tube flowmeter (P/N FM112–02C; Aalborg, Monsey, NY), which was used to set the flow rate of purified air delivered into the chamber containing the moths. Collector traps used to trap organic volatiles were made from a 4.0-cm-long by 4.0-mm-ID piece of glass tubing and contained 50 mg of Super-Q as the adsorbent (Analytical Research Systems, Gainesville, FL). Collector traps were connected to stainless steel tubing using 0.64-cm unions and 0.64-cm ID Teflon ferrules. These traps were cleaned by soxhlet extraction using methylene chloride for 24 h and dried in a fume hood before use. Volatiles were eluted using 100 μl of high purity methylene chloride. Before analysis, samples were concentrated with slightly elevated temperature.

Chemical Analysis. Chemicals obtained from excised glands and from volatile collections were analyzed by gas chromatography–mass spectroscopy (GC-MS). GC-MS analyses of samples were performed using a Thermo Trace GC coupled to a Finnigan GC/Q-MS. The GC was equipped with a 30-m by 0.25-mm ID DB-225MS column with 0.25-mm film thickness (Agilent, Palo Alto, CA). The initial oven temperature was held at 50°C for 2 min. It was increased at a rate of 10°C/min to 130°C, followed by a second ramp at a rate of 20°C/min to 210°C and held isothermal for 11 min. The injection port and ionizing source were kept at 150 and 170°C, respectively. Transfer-line temperature was held at 230°C. Injections were of 2 μl in volume using on-column injection. Column flow was set at 1.5 ml/min. There was a solvent delay of 6 min after which the mass spectrometer was activated. The mass spectra were collected from m/z 50.0–650.0 with signal averaging at a scan rate of 3 microscans/scan. Compound identifications were made by comparison of the mass spectra and retention times with those of corresponding reference samples from our library.

Lure Formulation. Single and multiple component lures were formulated using rubber septa (Heath et al. 1986) with a range of release rates and ratios (Heath et al. 1990). The synthetic lures that were used in most tests were formulated by USDA–ARS in Gainesville, FL. Additional lures were produced by an alternate source (Suterra, Bend, OR) for comparative purposes and were used in the last field test. A list of the lure formulations used in laboratory and field tests is given in Table 1.

Field Tests. Field tests were conducted in areas with endemic populations of cactus moth. An initial test was conducted in South Africa, and subsequent tests were conducted in Georgia and Florida or in Florida alone. Baited Pherocon 1-C Wing traps (Trécé, Adair, OK) were used in all field tests. Tests were conducted during peak flight periods. Treatments were traps baited with various release rates and/or ratios of the putative pheromone components tested in comparison with traps baited with two virgin females. Females were 1–2 d old when placed in the traps and were replaced after each sample period. Synthetic lures were replaced after 2 wk. Unless otherwise stated, number of males captured were determined every 3–4 d.

Field experiment 1 was conducted in South Africa from 1 to 8 November 2003 within a 100-ha cactus plantation. Two ratios of a three component blend, designated ARS-Blend A and ARS-Blend B1, were tested at doses of 10 μg, 100 μg, and 1 mg per septa. There were eight sets of traps each containing one of the seven treatments; virgin females; and three doses of two blends. Trap location within a replicate was randomly selected and rerandomized each day. Traps were wired onto cactus pads 0.5–1.0 m above ground along cactus rows. Each replicate set was separated by at least 20 m, and each trap within a replicate was 3–5 m apart. The number of males captured in each trap was determined daily. The remaining field experiments were conducted in Georgia and/or Florida. Traps were mounted on top of metal fence posts at a height of 1.5 m. Trap poles were 3 m apart and replicates (within a site) were separated by 10–20 m. Trap location was randomly selected at the beginning of each experiment and rotated each visit. Traps were serviced twice per week at alternating 3- and 4-d intervals, at which time the number of males captured was determined, females were replaced, trap bottoms were exchanged, and traps rotated within its replicate. Field experiment 2 was conducted in Florida and Georgia from 28 April 2004 to 8 May 2004 and tested the effect of dose on male capture. In this experiment, traps were baited with two virgin females or with 10 μg, 100 μg, or 1 mg ARS-Blend A lures. There were two replicates of all treatments tested in Florida and five replicates of all treatments tested in Georgia. Field experiment 3 was conducted in the

Table 1. Lure formulations of cactus moth female putative pheromone comprised of (*Z,E*)-9,12 tetradecadien-1-ol acetate (Z9,E12–14:Ac); (*Z,E*)-9,12 tetradecadien-1-ol (Z9,E12–14:OH); and (Z)-9-tetradecen-1-ol acetate (Z9-C14:Ac)

Lure[a]	Laboratory dose	Field test dose	Z9,E12–14:Ac	Z9,E12–14:OH	Z9-C14:Ac
Summer 2003[b]					
1 cmpt blend	1 μg, 10 μg		100%		
2 cmpt blend	100 ng, 1 μg, 10 μg		66.6%		33.3%
ARS-Blend A	100 ng, 1 μg, 10 μg		54%	42%	4%
Fall 2003[c]					
ARS-Blend A		10 μg, 100 μg, 1 mg	54%	42%	4%
ARS-Blend B1		10 μg, 100 μg, 1 mg	85%	5%	10%
Spring 2004					
ARS-Blend A		10 μg, 100 μg, 1 mg	54%	42%	4%
Summer 2004					
ARS-Blend A		10 μg, 100 μg, 1 mg, 10 mg	54%	42%	4%
Commercial-Blend A1		1 mg	54%	42%	4%
Fall 2004[d]					
Commercial-Blend A1		1 mg	54%	42%	4%
Commercial-Blend A2		1 mg	54%	42%	4%
Spring and Summer 2005					
Commercial-Blend A	10 μg[e]	1 mg	54%	42%	4%
Commercial-Blend B2	10 μg[e]	1 mg	60%	30%	10%
Commercial-Blend C	10 μg[e]	1 mg	30%	60%	10%
Commercial-Blend D	10 μg[e]	1 mg	70%	20%	10%

[a] Lures were formulated by ARS in Gainesville, FL (USDA–ARS, CMAVE) or commercially (Suterra, Bend, OR).
[b] Tested in olfactometer bioassays.
[c] Field test conducted in South Africa.
[d] Blends A1 and A2 have the same ratio blend, but a new source of (Z)-9-tetradecen-1-ol acetate was used for Blend A2.
[e] Males tested individually in flight tunnel bioassays.

same locations from 9 July 2004 to 20 August 2004 and tested the effect of dose and a comparison with a commercially formulated lure. In this experiment, traps were baited with the same treatments as the Spring 2004 test (Table 1) with the addition of traps baited with 10 mg ARS-Blend A and 1 mg commercial-Blend A1 lures. There were two replicates of this test in Florida and three replicates in Georgia. In field experiment 4, two versions of the commercial-Blend A were tested in field trials conducted from 6 to 18 October 2004. This test was necessitated because the synthetic lot of Z9-C14:Ac used for commercial-Blend A1 was no longer available and a new lot was used for formulation of commercial-Blend A2. There were five replicates of this test conducted in Florida, both lures were the 1-mg dose, and traps were checked daily. Field experiment 5 tested ratios of the three component blend (Table 1, Spring and Summer 2005) formulated at the 1-mg dose. This test was conducted from 19 April 2005 to 20 May 2005 and from 21 July 2005 to 25 August 2005. There were five replicates of this test in Florida during each of these time periods.

Statistical Analysis. In field tests, captures per replicate per treatment were summed over the entire sampling period, and cumulative capture was used in subsequent analysis. Effect of treatment was determined with one-way analysis of variance (ANOVA) using Proc GLM (SAS Institute 1985). Significant ANOVAs were followed by least significant difference (LSD) test ($P = 0.05$) for mean separation. The Box-Cox procedure, which is a power transformation that regresses log-transformed SDs ($y + 1$) against log-transformed means ($x + 1$), was used to determine the type of transformation necessary to stabilize the variance before analysis (Box et al. 1978).

Results and Discussion

Laboratory Mating Behavior. Individual virgin males were very active in the bioassay vials, with activity beginning several hours after the onset of scotophase and continuing periodically throughout the night. This activity included wing fanning while walking, and making short flights. Females assumed a typical calling posture (i.e., abdomen protruding upward through the wings) in six of the seven isolated females and in the remaining female that was paired with a male but for which no mating was observed. Among the isolated females, calling was initiated 2.0 ± 0.24 h before sunrise, but only 30 min before sunrise for the paired female that had not mated. Females remained in the calling posture until the laboratory lights were turned on ≈1–1.5 h after sunrise. Paired adults showed little courtship behavior, and in the small observation vials, mating occurred without the female engaging in overt calling behavior. Mating was observed in four of the seven pairs tested and was initiated ≈ 2–4 h before sunrise, with an average of 3.1 ± 0.70 h. Moths remained paired for 1.3–2.4 h, with average duration of 2.0 ± 0.40 h.

Unlike the females in the bioassay vials, females in the olfactometer bioassays did not initiate calling until ≈75 min before sunrise. Males placed in the olfactometer before calling was initiated did not respond until the female started calling, and response was almost instantaneous. Additional males added 60, 45, and 30 min before sunrise (and after the female started calling) also showed immediate response. Males responded in the olfactometer by wing fanning and moving up the tube to the side connected to the female. The male would rest on the screen separating

the female chamber from the main tube, or he would move away from the female and settle elsewhere in the olfactometer. All activity by the male was terminated within 15 min of placement in the olfactometer, and few responded when added to the olfactomer 15 min before sunrise.

Hight et al. (2003) documented mating behavior in the field. In tests using clipped-wing females on mating tables, females initiated calling an average of ≈45 min before sunrise, with a range of ≈80 to ≈20 min before sunrise. There was immediate response of males flying around the females and attempting copulation, although all successful copulations were initiated in a 15-min time period. As observed in the laboratory bioassays, there was little apparent courtship behavior and unmated females remained in a calling position until sunrise. Overall peak calling period in the laboratory correlated with calling period documented in the field by Hight et al. (2003), but the calling initiation occurred over a wider range of time in the laboratory.

Chemical Analysis. Initially, no candidate chemicals were observed in the GC-MS analyses of excised gland extracts or in volatile collections obtained from calling females. Changes in collection conditions such as time period of collection, number of females per sample, etc., found no difference between the chemical collection and the paired control sample. Similarly, there were no differences in volatile collections from time periods before the start of calling versus time periods when females assumed the calling posture. When PBAN was used to stimulate pheromone biosynthesis, however, three candidate components were obtained. These were identified as (*Z,E*)-9,12 tetradecadien-1-ol (Z9,E12–14:OH), (*Z,E*)-9,12 tetradecadien-1-ol acetate (Z9,E12–14:Ac), and (Z)-9-tetradecen-1-ol acetate (Z9-C14:Ac). Confirmation was obtained by comparing retention times and mass spectra of synthetic materials obtained from USDA–ARS in Gainesville, FL and Suterra. Retention times and spectra obtained using electron impact and chemical ionization (isobutane) were identical among the three sources of chemicals. The average ($n = 7$) percentages of Z9,E12–14:OH, Z9-C14:Ac, and Z9,E12–14:Ac were 42.3 ± 18.1, 3.7 ± 1.7, and 54.0 ± 18.8%, respectively.

Approximately 120 collections of volatile chemicals were made from 1- to 3-d-old virgin female moths. The number of females placed in the collection chamber varied from 20 to 50. Fourteen of these collections contained sufficient material for mass spectral analysis. Average percentages of Z9,E12–14:OH, Z9-C14:Ac, and Z9,E12–14:Ac were 29.6 ± 8.3, 8.5 ± 3.9, and 61.9 ± 7.1, respectively.

Laboratory Bioassays and Field Tests using Synthetic Compounds. Preliminary tests of male response to volatile chemicals from virgin females, from Z9E12–14:AC formulated as a single component, and from two- and three-component synthetic blends (Table 1; Summer 2003) were conducted in olfactometer bioassays. Males responded to volatile chemicals from calling females by wing fanning and moving up the olfactometer to the side with the female, with 8 of 11 males tested showing this response. Of five males tested for each dose/blend combination, only one moved to the side with the 1-μg single component lure and one moved to the side with the 10-μg two-component lure, but no wing fanning was observed in any of the responding or nonresponding males. However, three and four of five males per dose moved to the arm with volatiles from the 100-ng and 1-μg three-component ARS-Blend A lure, respectively, and wing fanning was observed in four of those males. Two of five males responded to the 10-μg three-component ARS-Blend A lure by wing fanning, but none moved up the arm with the lure.

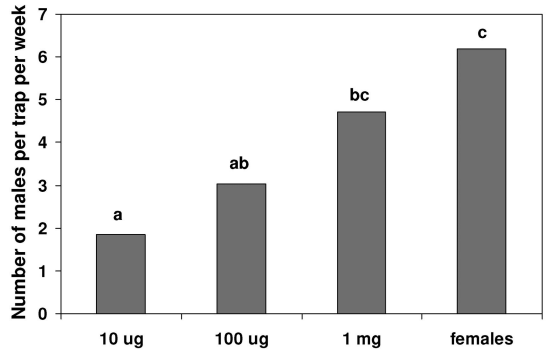

Fig. 1. Numbers of male cactus moths captured in Pheroconl-C Wing traps baited with two virgin females or with a three-component synthetic blend (ARS-Blend A) at one of three doses. Tests were conducted from 28 April 2004 to 8 May 2004 in areas with established populations in Florida and Georgia. Bars headed by the same letter are not significantly different ($P = 0.05$, LSD mean separation test).

Based on that preliminary data and preliminary chemical analyses, two ratios of the three-component blend were field tested in Africa (experiment 1, Fall 2003). ARS-Blend A, which was based on the ratio obtained from gland extracts, had approximately equal amounts of Z9,E12–14:OH and Z9,E12–14:Ac; ARS-Blend B1, which was based on the ratio obtained from volatile chemical collections, was predominantly Z9,E12–14:Ac. There were differences in number of males captured ($F = 22.69$; df = 6,35; $P < 0.0001$; log[x + 1] transformed data). Female-baited traps captured the greatest number of males per trap per week (6.5 ± 4.23), traps baited with 100 μg and 1 mg ARS-Blend A captured an intermediate number of males (0.8 ± 0.75 and 1.0 ± 0.89, respectively), and traps baited with 10 μg ARS-Blend A and any dose of ARS-Blend B1 captured the fewest males (capture ranged from 0.0 ± 0.00–0.2 ± 0.41).

In field experiment 2 (Spring 2004), there was an effect of treatment on capture of males ($F = 10.25$, df = 3,24; $P = 0.0002$). Capture in traps baited with the 1-mg dose of ARS-Blend A lure was statistically equal to that in traps baited with virgin females, and there was a direct relationship between synthetic lure dosage and male capture (Fig. 1). Experiment 3 (Summer 2004) determined if an increase in synthetic lure dosage would further increase capture and tested a com-

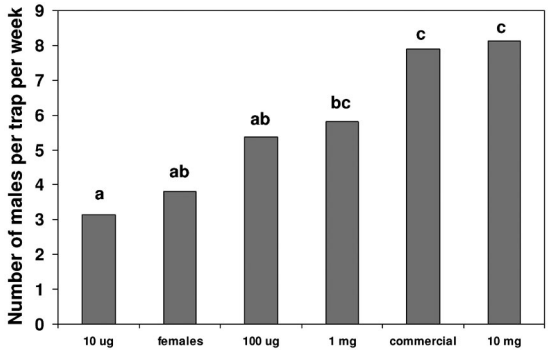

Fig. 2. Numbers of male cactus moths captured in Pherocon 1-C Wing traps baited with two virgin females, with lures containing a three-component synthetic blend (ARS-Blend A) at one of four doses or with a commercially produced version of the lure (commercial-Blend A) formulated at 1 mg. Tests were conducted from 9 July 2004 to 20 August 2004 in areas with established populations in Florida and Georgia. Bars headed by the same letter are not significantly different ($P = 0.05$, LSD mean separation test).

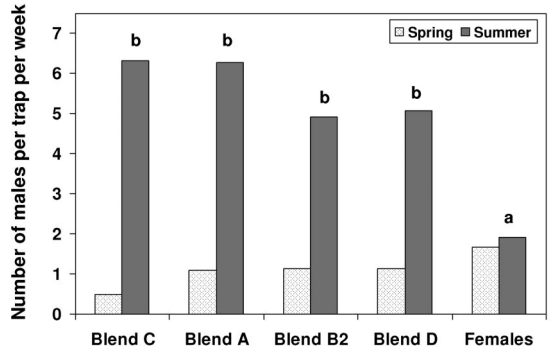

Fig. 3. Numbers of male cactus moths captured in Pherocon 1-C Wing traps baited with two virgin females, with lures containing a three-component synthetic blend formulated at 1 mg but with different ratios of the three components (Table 1). Tests were conducted from 19 April 2005 to 20 May 2005 (spring, lighter bars) and from 21 July 2005 to 25 August 2005 (summer, darker bars) in areas with established populations in Florida. Bars headed by the same letter are not significantly different ($P = 0.05$, LSD mean separation test on square root [x + 0.5]-transformed data, nontransformed means presented).

mercial formulation of the 1-mg Blend A lure. Again, there was a direct relationship between lure dose and male capture (Fig. 2), and capture in traps baited with any of the synthetic lures was equal to or better than capture in traps baited with virgin females ($F = 6.28$; df = 5,24; $P = 0.0007$). Capture in traps baited with the synthetic lure produced by the commercial company (commercial-Blend A1) was equal in effectiveness to traps baited with synthetic lures produced by us. Capture of nontarget moth species was also recorded during this test, and traps baited with live females, with the commercial-Blend A1, and with 1 and 10 mg ARS-Blend A captured males of the beet armyworm, *Spodoptera exigua* (Hübner) (Noctuidae); and traps baited with the Commercial-Blend A1 and with 10 mg ARS-Blend A lures captured males of the grapeleaf skeletonizer, *Harrisina americana* (Guerin) (Zygaenidae). Z9,E12–14:Ac has been reported as a single pheromone component and in combination with Z9,E12–14:OH and Z9–C14:Ac as 3 of 11 components reported for the beet armyworm (Brady and Ganyard 1972 and Tumlinson 1981, respectively). None of these compounds has been reported for the grapeleaf skeletonizer, but all three components have also been reported in putative pheromone blends for the Indianmeal moth, *Plodia interpunctella* (Hübner), the almond moth, *Cadra cautella* (Walker), and *Myelois cribrella* Hübner (Witzgall et al. 2004), three other pyralid species.

The third flight period for the cactus moth in Florida and Georgia occurs in the fall, and additional commercial lures were needed for this test. The synthetic lot of (Z)-9-tetradecen-1-ol acetate used for the initial production was depleted, and a new source was used for the new batch, designated commercial-Blend A2. Experiment 4, a field test conducted in Fall 2004, determined if change in synthetic source would affect lure performance and evaluated lure efficacy for the fall population peak. There were no differences in captures in traps baited with either formulation or with females ($F = 0.10$; df = 2,14; $P = 0.9036$; log[x + 1] transformed) and average capture was 0.9 ± 0.51 males per trap per week over all treatments.

Experiment 5 evaluated changes in the ratio of the three-component blend in comparison with Blend A. Blends C, B2, and D contained more Z9-C14:Ac (10% versus 4% in Blend A), and the remaining 90% was comprised of 30, 60, and 70% Z9,E12–14:Ac and 60, 30, and 20% Z9,E12–14:OH, respectively. In the field test conducted in Spring 2005, traps baited with females tended to capture the most and traps baited with Blend C tended to capture the fewest males (Fig. 3, lighter bars), but the differences were not significant ($F = 1.49$; df = 4,20; $P = 0.2431$). Populations were very low during this test, so the study was repeated during the Summer 2005 flight. During that test, traps baited with any of the three-component blends captured more males than traps baited with females ($F = 2.95$; df = 4,20; $P = 0.0457$; Fig. 3, darker bars). The numbers of males captured in female-baited traps were approximately equal during the spring and summer tests; however, the female-baited traps were the best during the spring test but the poorest during the summer test. It is not known if discrepancies obtained during these two tests were caused by changes in viability of females in traps, to differences in male population/response during these two flight periods, or to variations in environmental conditions in spring versus summer. Therefore, flight tunnel tests were conducted to compare directly male behavioral response to live females versus the different synthetic blends to better understand underlying responses to the different lure-baited traps.

In the flight tunnel bioassays, males responded to females placed inside the tunnel in cages and to vol-

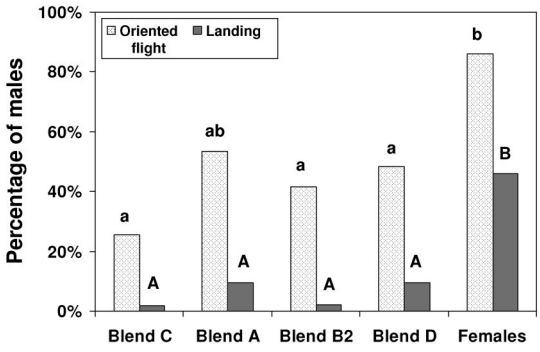

Fig. 4. Percentage of 20 cactus moth males per test that responded with oriented flight (lighter bars) and/or landing (darker bars) to live females or to lures containing a three-component synthetic blend formulated at 10 μg but with different ratios of the three components (Table 1) in laboratory flight tunnel assays. All tests were conducted during the last hour of scotophase. Bars headed by the same letter within a response variable are not significantly different ($P = 0.05$, LSD mean separation test; percentage landing data were square root [x + 0.5]-transformed, nontransformed means presented).

atile chemicals piped in from females placed outside of the tunnel. After oriented flight, some males landed on the cage or tube and, once in contact with the cage containing females, the male would walk and wing fan over the exterior surface. Percentage displaying oriented flight was highest in males responding to live females, lowest in males responding to synthetic Blends C, B2, and D; and intermediate in males responding to Blend A (Fig. 4, lighter bars). Percentage of males landing on the odor source was higher for live females than for any of the synthetic blends, and there were no differences among any of the blend ratios (Fig. 4, darker bars). Thus, it seems that identification of additional pheromonal components is needed to fully elucidate the pheromone of the cactus moth and/or presentation of the odor source and release rates and ratios tested may have provided a more complete response by male moths.

In recent studies, Pophof et al. (2005) measured the electrophysiological response of cactus moth olfactory sensilla to 14 chemicals identified as pheromone components for other pyralid species and 62 putative volatile chemicals from cactus plants. On a scale of 0 (no response) to 3 (strongest response), they rated response to Z9,E12–14:Ac as a 3, and this was the only pheromonal component tested that rated a 3. Responses to Z9-C14:Ac along with other alcohols and an acetate were rated as 1 (weak response). Z9,E12–14:OH was not tested in that study, so relative strength of electrophysiological response to this compound in comparison with the other components is unknown.

The three-component synthetic blend reported here has been shown to attract male cactus moths in flight tunnel bioassays, in olfactometer bioassays, and in field tests. Thus, an experimental attractant for cactus moth males has been developed that has a 1-mg load of the three-component blend of 54% Z9,E12–14:Ac, 42% Z9, E12–14:OH, and 4% Z9-C14:Ac. Changes in the ratio of these components had little effect on lure efficacy. This attractant is based on chemical components produced in the abdominal gland and emitted from calling females. However, only small amounts of actual pheromone are released from calling females, most likely several orders of magnitude less than the amount released from a septa loaded with 1 mg of synthetic chemicals. Roelofs (1978) hypothesized that there is an interaction between isomer ratio and dosage that affects male attraction such that high dose of incorrect blends may result in capture equal to that of low dose of correct blends. Tumlinson et al. (1986) noted that it is possible to capture males of a species with an incorrect or incomplete pheromone blend or even with an analog of a pheromone component, particularly when massive doses are used. The 1-mg load used for the cactus moth experimental attractant is a "massive dose." Thus, identification of additional components is needed to elucidate all the pheromonal components used by cactus moth females. Addition of these components and possible changes in release rate and/or ratio may further improve effectiveness of the lure. Availability of further optimized lures may be needed for detection of newly invading moths in areas currently free of this pest, because effectiveness of this lure for population detection and delimitation has not yet been determined. Nevertheless the three-component experimental attractant can be used for monitoring populations of cactus moth males and the use of this lure for capture of male cactus moths from known populations has been shown.

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